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Design and synthesis of novel neuroprotective 1,2-dithiolane/chroman hybrids

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ABSTRACT

Novel 1,2-dithiolane/chroman hybrids bearing heterocyclic rings such as 1,2,4- and 1,3,4-oxadiazole, 1,2,3-triazole and tetrazole were designed and synthesized. The neuroprotective activity of the new analogues was tested against oxidative stress-induced cell death of glutamate-challenged HT22 hippocampal neurons. Our results show that bioisosteric replacement of amide group in 2-position of the chroman moiety, by 1,3,4-oxadiazole did not affect activity. However, analogue **5** bearing the 1,2,4-oxadiazole moiety showed improved neuroprotective activity. The presence of nitrogen heterocycles strongly influences the neuroprotective activity of 5-substituted chroman derivatives, depending on the nature of heterocycle. Replacement of the amide group of the first generation analogues by 1,2,4-oxadiazole or 1,2,3-triazole resulted in significant improvement of the activity against glutamate induced oxidative stress.

1. Introduction

An increasing number of studies published the last decade have reported that α-lipoic acid (LA) is beneficial in a number of oxidative stress models of cell death. The potential of LA to work as a therapeutic agent appears to lie not only in its actions as a direct scavenger of ROS/RNS, but also in its ability to affect signaling cascades.[1]

In addition, LA represents an ideal chemical entity for the development of multifunctional compounds.[2] The design and synthesis of hybrid molecules encompassing two pharmacophores in one molecular scaffold is a well established approach to the synthesis of more potent drugs with dual activity.[3] Using this approach, several research groups have recently designed and synthesized hybrid molecules by coupling LA with other bioactive molecules. These efforts resulted in new molecules with antioxidant activity hyphenated with a wide variety of other activities such as: protection against reperfusion arrhythmias,[4,5] nitric oxide synthase inhibition,[6] erythrocyte protection from hemolysis,[7] antiproliferative activity,[8,9] acetylcholinesterase inhibition,[10] butyrylcholinesterase inhibition,[11] as well as radioprotective,[12] and anti-inflammatory activity.[13,14]

Moreover, combination of 1,2-dithiolane-3-alkyl group with the catechol moiety through five-membered heterocyclic rings, as bioisosters,[15] of the amide bond led to compounds which exhibited strong neuroprotective activity.[16]

Based on our experience on bioactive dithiolane analogues, we designed and synthesized novel 1,2-dithiolane/chroman hybrids containing heterocyclic rings such as 1,2,4- and 1,3,4-oxadiazole, 1,2,3-triazole and tetrazole. The neuroprotective activity of the new analogues was evaluated using glutamate-challenged hippocampal HT22 cells.

2. Chemistry

The synthesis of 1,2,4-oxadiazole analogues is depicted in Scheme 1. For the preparation of racemic 2-substituted chroman analogue **5**, mesylate **1**[16] was converted to nitrile **2** using NaCN, DMSO and then, to the *N*-hydroxy-amidine **3** by treatment with hydroxylamine hydrochloride and Et_3N. Reaction of **3** with *N*-hydroxysuccinimidyl-trolox ester gave the acyl amidoxime **4** and subsequent intramolecular cyclization in the presence of tetrabutylammonium fluoride produced the 1,2,4-oxadiazole analogue **5**. 5-Substituted chroman analogue **10** was prepared from bromide **6**[5] using similar procedure and was deprotected using $BF_3{\cdot}S(Me)_2$[17] to afford the final analogue **11**.

1,3,4-Oxadiazole analogues were synthesized as shown in Scheme 2. Hydrazide **12** was prepared from the trolox methyl ester and then reacted with *N*-hydroxysuccinimide-activated LA to give **13**. Cyclodehydration in boiling $POCl_3$ produced the 2-substituted chroman analogue **14**. For the synthesis of 5-substituted chroman derivatives, LA was converted to the corresponding ethyl ester **15** and then to hydrazide **16** by treatment with hydrazine. Reaction of **16** with 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carboxylic acid in the presence of benzotriazol-1-

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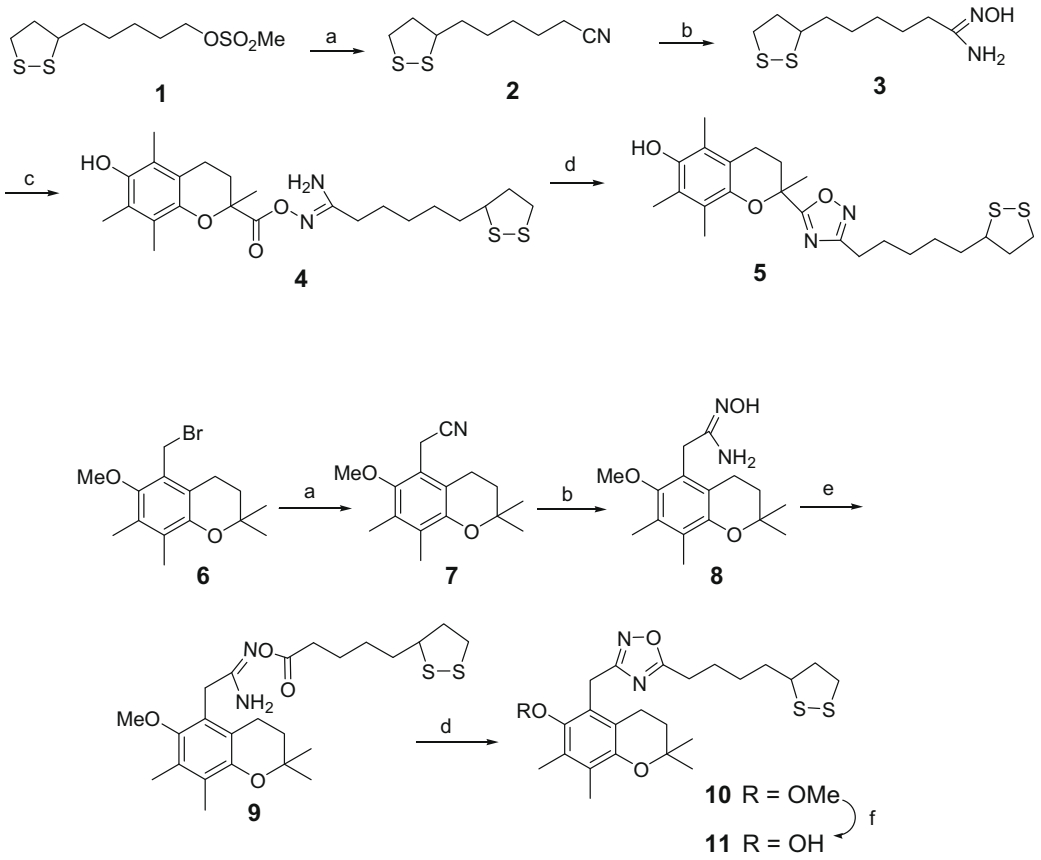


Scheme 1. Synthesis of 1,2,4-oxadiazole analogues. Reagents and conditions: (a) NaCN, DMSO; (b) $NH_2OH{\cdot}HCl$, Et_3N, EtOH, (c) *N*-hydroxysuccinimidyl-trolox ester, CH_2Cl_2; (d) TBAF, THF; (e) lipoic acid, DCC, CH_2Cl_2; (f) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.

yloxytris(dimethylamino) phosphonium hexafluorophosphate (BOP) and Et_3N, gave hydrazide **17** which was cyclized in boiling $POCl_3$ to afford **18**. Analogue **19** was obtained by treatment of **18** with $BF_3{\cdot}S(Me)_2$.

Cu^I-catalyzed 'click' cycloaddition[18–20] between 3-(5-azidopentyl)-1,2-dithiolane and alkynes **23, 27**, **28** as well as between (3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)-methylazide, and alkyne **34**, in the presence of $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, afforded the 1,2,3-triazole analogues **24, 29**, **30**, **35** (Scheme 3).

Reduction of trolox ethyl ester **20** to the alcohol **21,** followed by Swern oxidation[21,22] gave aldehyde **22** which, in turn reacted with Bestmann–Ohira reagent[23,24] in the presence of K_2CO_3 to afford alkyne **23**. Alkynes **27** and **28** were prepared by treatment of the appropriate aldehydes[25,26] with Bestmann–Ohira reagent in the presence of K_2CO_3. Alkyne **34** was prepared from lipolol which was oxidized to the corresponding aldehyde **33** using Pfitzner–Moffatt conditions.[27,28]

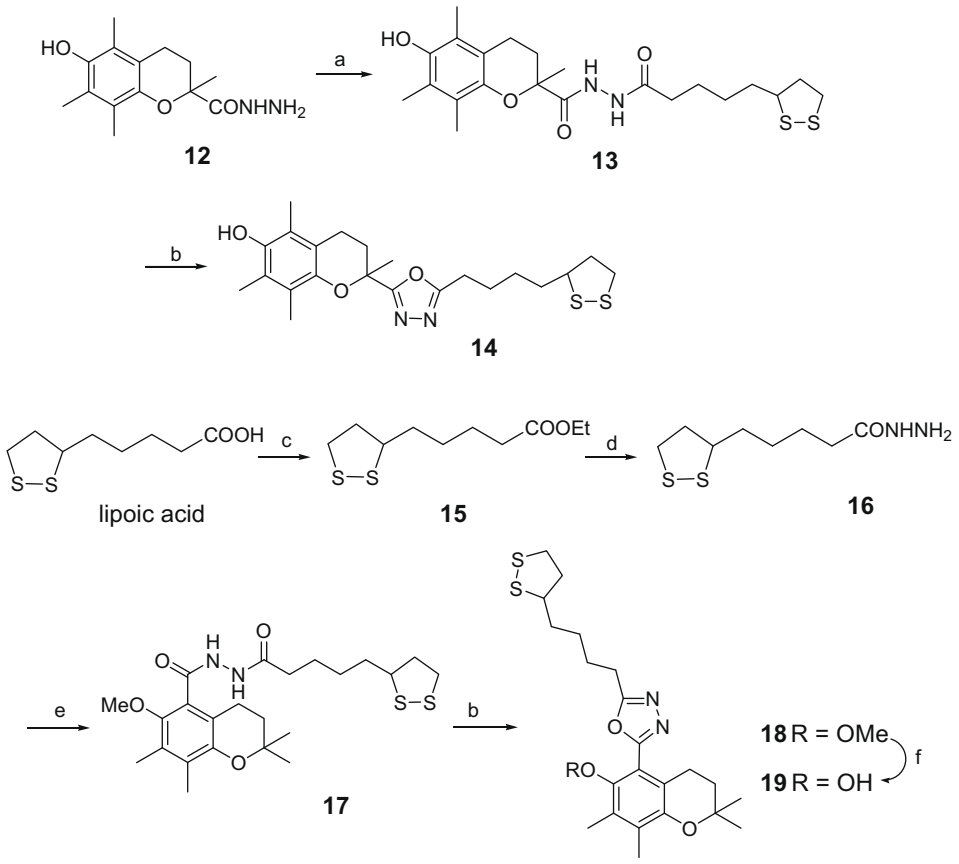
The synthesis of tetrazole derivatives is depicted in Scheme 4. Acylation of amines **36** and **37** with *N*-(lipoyloxy)succinimide, according to procedures reported in previous publications of our group,[5,26] afforded amides **38** and **39**, which in turn were converted to thioamides **40** and **41** by treatment with Lawesson's reagent. Tetrazoles **42** and **43** were obtained by treatment of thioamides with trimethylsilyl azide ($TMSN_3$), in the presence of triphenylphosphine and diisopropylazodicarboxylate (DIAD).[29] Analogue **44** was obtained by treatment of **42** with $BF_3{\cdot}S(Me)_2$.

Amide analogues **I** and **II** which were previously synthesized in our laboratory[5] as well as compound **45**, synthesized from activated trolox and 5-(1,2-dithiolan-3-yl)pentanamine, were used as controls in order to investigate the influence of the replacement of amide group by nitrogen heterocycles on the activity against oxidative stress-induced cell death in HT22 cells.

3. Results and discussion

The mouse hippocampal cell line HT22 has been used to elucidate sequential cellular events during programmed cell death from oxidative stress (oxytosis) caused by glutamate-induced depletion of intracellular glutathione.[30] Although HT22 cells lack ionotropic glutamate receptors that could mediate excitotoxicity, they undergo oxytosis within 24 h following exposure to 1–5 mM glutamate. Recent findings suggest that oxytosis faithfully mimics cytotoxicity due to oxidative stress in Alzheimer's disease and other neurodegenerative disorders.[31,32]

Figure 1 shows the neuroprotective activity of the 2-substituted chroman derivatives against oxytosis of glutamate-challenged hippocampal HT22 cells. Amide analogue **I** and its isostere **45**, have comparable EC_{50} values (1.24 ± 0.38 and 1.59 ± 0.53 μM, respectively) (Table 1, column 2). Bioisosteric replacement of amide group of analogue **45** by 1,3,4-oxadiazole (analogue **14**, $EC_{50} = 1.65 \pm 0.36$ μM) had no effect in antioxidant activity. However, analogue **5** bearing the 1,2,4-oxadiazole moiety showed improved neuroprotective activity ($EC_{50} = 0.93 \pm 0.19$ μM). Differences in activity between 1,2,4-oxadiazoles and 1,3,4-oxadiazoles were also observed by other researchers.[33] Specifically, C-glucosylated 1,3,4-oxadiazoles proved practically inactive while the 1,2,4-oxadiazole series displayed inhibition of glycogen phosphorylase in the micromolar range. Although in this case the nature of the heterocycle influences the interaction with the enzyme and thus the inhibitory



Scheme 2. Synthesis of 1,3,4-oxadiazole analogues. Reagents and conditions: (a) *N*-(lipoyloxy)succinimide, CH_2Cl_2; (b) $POCl_3$, 100 °C; (c) $SOCl_2$, EtOH; (d) $NH_2NH_2{\cdot}H_2O$, MeOH; (e) 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-1-benzopyran-5-carboxylic acid, BOP, Et_3N, DMF, CH_2Cl_2; (f) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.

activity, prevention of oxytosis of HT22 cells depends on inhibition of the increase in ROS production caused by the decrease of cellular GSH level upon treatment with glutamate.[30]

In general, apart from **24**, the 2-substituted derivatives were fully effective against oxytosis at concentrations $\geqslant 3$ μM (Fig. 1 and Table 1, column 4).

The presence of nitrogen heterocycles strongly influences the neuroprotective activity of 5-substituted chroman derivatives (Fig. 2 and Table 1). Replacement of the amide group of analogue **II** ($EC_{50} = 2.10 \pm 0.40$ μM) by 1,2,4-oxadiazole or 1,2,3-triazole resulted in significant improvement of the activity against glutamate induced oxidative stress. Thus, analogues **11** and **31** exhibited $EC_{50} = 0.57 \pm 0.10$ and 0.90 ± 0.04 μM, respectively. The 1,3,4-oxadiazole analogue **19,** in which the heteroaromatic ring is directly connected to the chroman moiety, was weakly effective at the concentrations tested (Fig. 2, Table 1, column 4). The presence of methylene group between the chroman and the five membered rings seems to increase the activity of these hybrids. Moreover, the less flexible among these analogues, tetrazole **44** was less active ($EC_{50} = 3.04 \pm 1.15$ μM) than the amide counterpart **II**.

4. Conclusions

Our results show that the bioisosteric replacement of the amide group by five-membered nitrogen heterocycles influences the neuroprotective activity of the new compounds, in a manner depending on the position of the substitution as well as the nature of heterocycle. Thus, the 2-substituted analogue **5** and the 5-substituted analogue **11**, both bearing 1,2,4-oxadiazole rings, exhibited the strongest neuroprotective activity, with **11** being ~2 times more potent than **5** (Table 1, column 3). Similarly, the 5-substituted chroman **31** bearing 1,2,3-triazole moiety was active at low μM concentrations, while the 2-substituted analogue **24**, bearing the same moiety, was 10 times less potent (Table 1, column 3) and only weakly effective at 10 μM (Table 1, column 4).

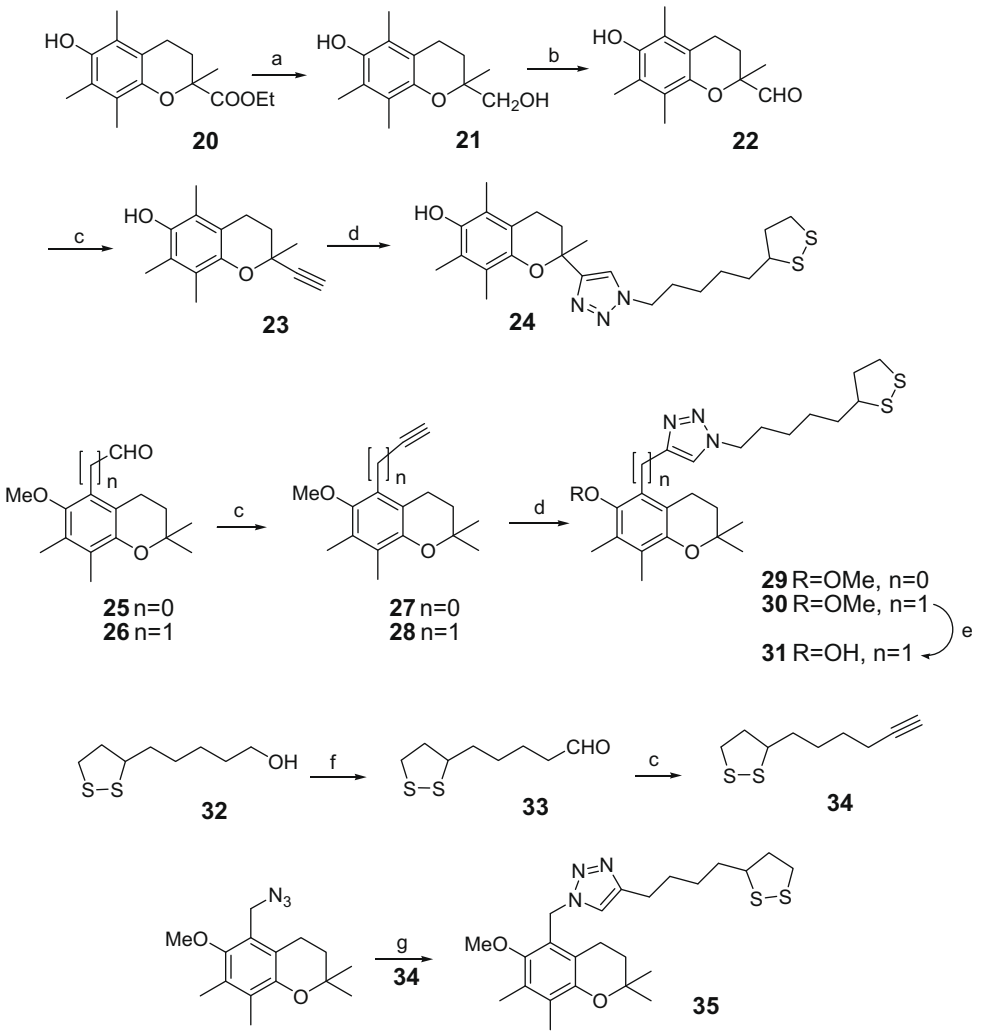
5. Experimental section

5.1. Chemistry

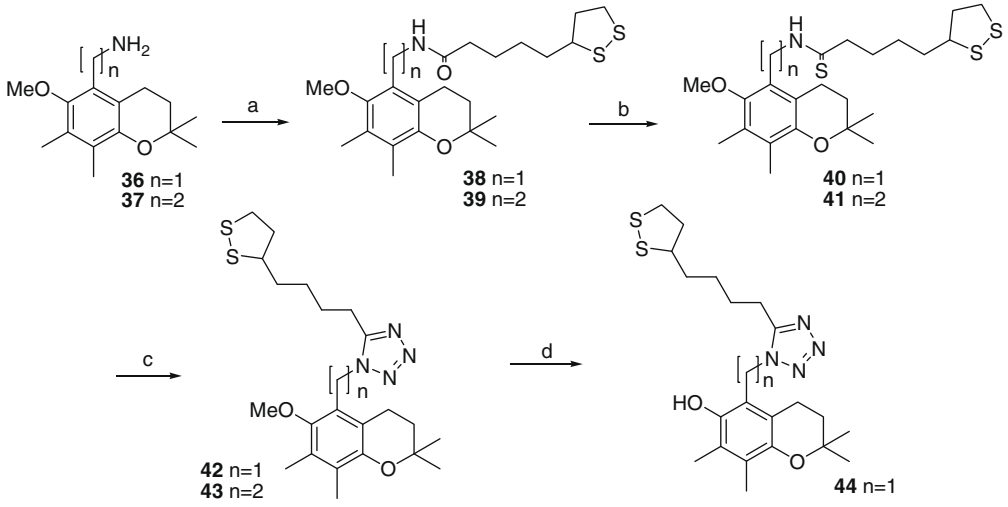
Melting points were determined on a Buchi 510 apparatus and are uncorrected. NMR spectra were recorded on a Varian 300 spectrometer operating at 300 MHz for ^1H and 75.43 MHz for ^{13}C spectra with $CDCl_3$ as solvent. Silica gel plates Macherey–Nagel Sil G-25 UV_{254} were used for thin layer chromatography. Chromatographic purification was performed with silica gel (200–400 mesh). Mass spectra were recorded on TSQ 7000 Finigan instrument in the ESI mode. HRMS were in FAB mode.

5.1.1. 1,2-Dithiolan-3-hexanenitrile (2)

To a solution of analogue **1** (0.100 g, 0.37 mmol) in 2 mL anhyd DMSO, was added NaCN (0.091 g, 1.85 mmol) and the mixture was stirred at rt overnight. After completion of the reaction, cold water was added and the mixture was extracted with Et_2O, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated and the residue was purified by flash-column chromatography (pet. ether/AcOEt 80:20). Yield: 0.050 g (66%), yellow gummy solid. ^1H NMR δ: 3.73–3.68 (m, 1H), 3.24–3.20 (m, 1H), 2.98–2.89 (m, 2H), 2.62–2.58 (m, 1H), 2.33 (t, $J = 7.0$ Hz, 2H), 1.79–1.60 (m, 4H), 1.49–1.41 (m, 2H), 1.39–1.21 (m, 2H). ^{13}C NMR δ: 119.9, 42.6, 39.2, 34.0, 28.4, 25.9, 25.5, 22.0, 17.3.



Scheme 3. Synthesis of 1,2,3-triazole analogues. Reagents and conditions: (a) $LiAlH_4$, THF, 70 °C; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −60 °C; (c) Bestmann–Ohira reagent, K_2CO_3, CH_3OH; (d) 3-(5-azidopentyl)-1,2-dithiolane, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH, H_2O; (e) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2; (f) DMSO, *N,N′*-diisopropyl carbodiimide, $Cl_2CHCOOH$; (g) $CuSO_4{\cdot}5H_2O$, sodium ascorbate, *t*-BuOH, H_2O.



Scheme 4. Synthesis of tetrazole analogues. Reagents and conditions: (a) *N*-(lipoyloxy)succinimide, CH_2Cl_2; (b) Lawesson's reagent, THF; (c) $TMSN_3$, DIAD, Ph_3P, THF; (d) $BF_3{\cdot}S(Me)_2$, CH_2Cl_2.

5.1.2. *N*-Hydroxy-1,2-dithiolan-3-hexamidine (3)

To a solution of nitrile **2** (0.050 g, 0.244 mmol) in 4 mL abs EtOH, were added Et_3N (0.1 mL), $NH_2OH{\cdot}HCl$ (0.085 g, 1.22 mmol) and the mixture was stirred at 40 °C overnight. The mixture was then diluted by AcOEt, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated

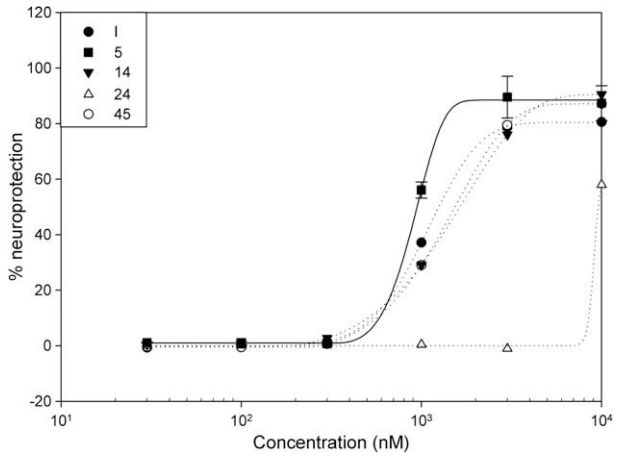


Figure 1. Protection of HT22 cells from oxidative stress-induced cell death by 2-substituted chroman analogues. Cells were challenged with 5 mM glutamate in the absence or presence of increasing concentrations of the hybrids for 24 h and relative numbers of viable cells were assessed as described in experimental section. Values are mean ± SEM (shown only for **5**) of three independent experiments.

and the residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 95:5). Yield: 0.030 g (52%), green, gummy solid. ^{1}H NMR δ: 4.52 (br s, 1H, –O*H*), 3.74–3.66 (m, 1H, –*CH*SS–), 3.27–3.18 (m, 1H, –*H*CHCH$_2$SS–), 2.99–2.91 (m, 2H, –C*H*$_2$SS–), 2.62–2.56 (m, 1H, –HC*H*CH$_2$SS–), 2.15 (t, *J* = 7.0 Hz, 2H, –C*H*$_2$C=NOH), 1.74–1.20 (m, 8H, (C*H*$_2$)$_4$).

5.1.3. *N*-(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-carbonyloxy)-1,2-dithiolan-3-hexanamidine (4)

To a solution of analogue **3** (0.035 g, 0.15 mmol) in 3 mL anhyd CH_2Cl_2, was added a solution of trolox (0.037 g, 0.15 mmol) and DCC (0.031 g, 0.15 mmol) in 3 mL anhyd CH_2Cl_2 and the mixture was stirred at rt overnight. CH_2Cl_2, was then added and the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, evaporated to dryness and the residue was purified by flash-column chromatography (pet. ether/AcOEt 70:30). Yield 0.020 g (28%), yellow gummy solid. ^{1}H NMR δ: 4.05 (br s, 1H), 3.76–3.62 (m, 1H), 3.27–3.18 (m, 1H), 2.99–2.83 (m, 2H), 2.64–2.52 (m, 4H), 2.20 (s, 3H, Ar*Me*), 2.17 (s, 3H, Ar*Me*), 2.15–2.09 (m, 2H), 2.06 (s, 3H, Ar*Me*), 1.92–1.82 (m, 1H), 1.75–1.48 (m, 11H). ^{13}C NMR δ: 171.7, 159.8, 146.3, 145.5, 122.0, 121.6, 119.3, 117.9, 78.1, 42.9, 39.3, 34.0, 31.3, 28.8, 26.8, 26.5, 26.1, 22.2, 22.0, 21.3, 12.5, 12.0, 11.5. MS *m/z*: 467.3 (M+H)$^+$.

5.1.4. 3-(6-(1,2-Dithiolan-3-yl)pentyl)-5-(2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran))-1,2,4-oxadiazole (5)

To a solution of analogue **4** (0.015 g, 0.031 mmol) in 2 mL anhyd THF was added *n*-TBAF and the mixture was stirred at rt for 2 h. The solvent was then evaporated and the residue was diluted in AcOEt. The organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated and the residue was purified by flash-column chromatography (pet. ether/AcOEt 70:30). Yield: 0.007 g (50%), yellow gummy solid. ^{1}H NMR δ: 4.29 (br s, 1H), 3.75–3.66 (m, 1H), 3.29–3.19 (m, 1H) 2.99–2.85 (m, 2H), 2.71–2.57 (m, 5H), 2.21 (s, 3H), 2.17 (s, 3H), 2.06 (s, 3H), 1.77–1.56 (m, 13H). MS *m/z*: 449.8 (M+H)$^+$.

5.1.5. 2-(3,4-Dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-benzopyran-5-yl)acetonitrile (7)

To a solution of 2-(6-methoxy-2,2,7,8-tetramethylchroman-5-yl)methyl bromide **6** (0.080 g, 0.26 mmol) in 2 mL anhyd DMSO, NaCN (0.063 g, 1.28 mmol) was added at 0 °C and the mixture was stirred at rt for 24 h. After completion of the reaction cold water was added and the mixture was extracted with Et_2O. The organic layer was washed with satd aqueous NaCl, dried over Na_2SO_4, filtered and the solvent was evaporated in vacuo. Purification by flash-column chromatography (pet. ether/AcOEt 80:20) afforded compound **7** as yellowish gummy solid. Yield: 0.050 g (75%) ^{1}H NMR δ: 3.73 (s, 3H, O*Me*), 3.70 (s, 2H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.20 (s, 3H), 2.11 (s, 3H), 1.84 (t, *J* = 6.8 Hz, 2H), 1.32 (s, 6H).

5.1.6. *N*′-Hydroxy-2-(3,4-dihydro-6-methoxy-2,5,7,8-tetramethyl-2*H*-benzopyran-5-yl) acetamidine (8)

To a solution of compound **7** (0.080 g, 0.31 mmol) in 6 mL abs EtOH, were added $NH_2OH{\cdot}HCl$ (0.107 g, 1.54 mmol), Et_3N (0.22 mL, 1.54 mmol) and the mixture was stirred at 60 °C for 24 h. After completion of the reaction, AcOEt and satd aqueous NaCl were added, the organic layer was dried and the solvent evaporated. The residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 95:5). Yield 0.060 g (66%) grey solid, mp 167–171 °C. ^{1}H NMR δ: 4.93 (br s, 2H), 3.69 (s, 3H), 3.45 (s, 2H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.20 (s, 3H), 2.09 (s, 3H), 1.74 (t, *J* = 6.8 Hz, 2H), 1.27 (s, 6H). ^{13}C NMR δ: 149.6, 148.7, 148.6, 128.2, 125.4, 124.0, 118.4, 73.3, 61.0, 32.9, 28.5, 27.0, 20.3, 13.1, 12.2. MS *m/z*: 293.5 (M+H)$^+$.

5.1.7. *N*′-(5-(1,2-Dithiolan-3-yl)pentanoyloxy)-2-(6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl) acetamidine (9)

To a solution of analogue **8** (0.040 g, 0.137 mmol) in 4 mL anhyd CH_2Cl_2, were added lipoic acid (0.028 g, 0.137 mmol) and DCC (0.034 g, 0.164 mmol) and the mixture was stirred at rt for 24 h. CH_2Cl_2 and satd aq were then added, the organic layer was dried and the solvent evaporated. Purification by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3) afforded **9** as green-yellow solid, mp 109–111 °C. Yield 0.042 g (63%) ^{1}H NMR δ: 5.18 (br s, 2H), 3.69 (s, 3H), 3.54 (m, 3H), 2.78 (t, *J* = 6.5 Hz, 2H), 2.46–2.35 (m, 3H), 2.18 (s, 3H), 2.08 (s, 3H), 1.94–1.85 (m, 2H), 1.77–1.67 (m, 7H), 1.49–1.47 (m, 2H), 1.26 (s, 6H). ^{13}C NMR δ: 171.2, 158.1, 149.5, 148.9, 128.3, 125.9, 123.4, 118.7, 73.4, 61.0, 56.5, 40.4, 38.7, 34.8, 33.2, 32.8, 29.0, 28.3, 27.0, 26.6, 25.0, 20.5, 13.1, 12.3. MS *m/z*: 481 (M+H)$^+$.

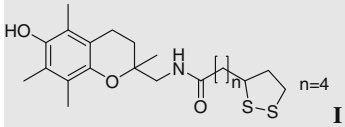
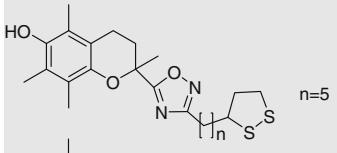
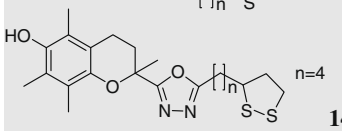
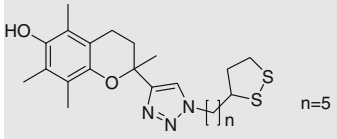
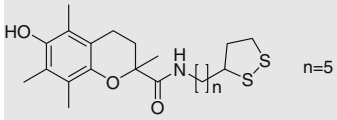
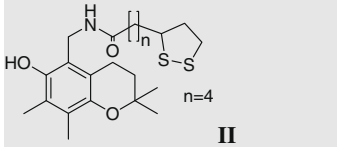
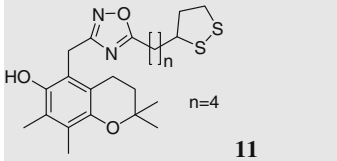
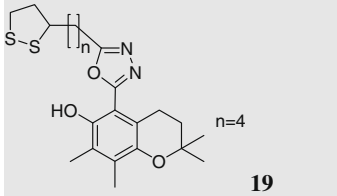
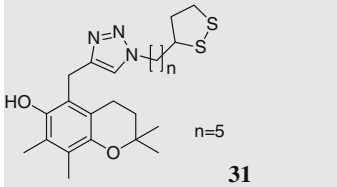
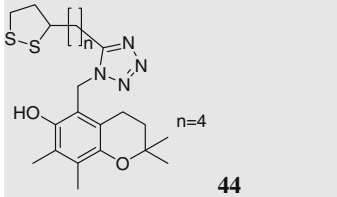
5.1.8. 5-(4-(1,2-Dithiolan-3-yl)butyl)-3-((3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1,2,4-oxadiazole (10)

To a solution of analogue **9** (0.040 g, 0.083 mmol) in 2 mL anhyd THF, was added TBAF (0.08 mL, 0.083 mmol) and the mixture was stirred at rt for 30 min. The solvent was then evaporated and the residue was taken up by AcOEt. The organic layer was washed with satd aqueous NaCl, dried over Na_2SO_4, filtered and the solvent was evaporated in vacuo. Purification by flash-column chromatography (pet. ether/AcOEt 70:30) gave **10** as yellowish gummy solid. Yield 0.035 g (92%). ^{1}H NMR δ: 4.09 (s, 2H), 3.68 (s, 3H), 3.57–3.52 (m, 1H), 3.18–3.10 (m, 2H), 2.84 (t, *J* = 7.5 Hz, 2H), 2.65 (t, *J* = 6.7 Hz, 2H), 2.47–2.41 (m, 1H), 2.20 (s, 3H), 2.10 (s, 3H), 1.94–1.67 (m, 7H), 1.57–1.46 (m, 2H), 1.28 (s, 6H). ^{13}C NMR δ: 179.3, 169.7, 149.7, 148.2, 128.2, 125.4, 123.4, 117.7, 73.0, 61.2, 56.4, 40.4, 38.7, 34.6, 33.0, 28.8, 27.1, 26.6, 26.5, 23.8, 20.7, 13.1, 12.3. MS *m/z*: 463.3 (M+H)$^+$. HRMS calcd for $C_{24}H_{34}O_3N_2S_2$: 462.2011, found: 462.2019.

5.1.9. 5-(4-(1,2-Dithiolan-3-yl)butyl)-3-((3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1,2,4-oxadiazole (11)

To a solution of **10** (0.030 g, 0.064 mmol) in 2 mL anhyd CH_2Cl_2, $BF_3{\cdot}S(Me)_2$ (0.1 mL, 1 mmol) was added and the mixture was stirred at ambient temperature overnight. The solvent was evaporated under argon and the residue was extracted with ethyl acetate and water. The organic layer was washed with saturated aqueous NaCl,

Table 1
Efficacy and potency of dithiolane/chroman hybrids to protect glutamate-challenged HT22 cells from oxytosis

Compound	EC_{50}[a] (μM) (mean ± SEM)	Relative potency[b]	Efficacy[c] (%)
 **I**	1.24 ± 0.38	1	80
 **5**	0.93 ± 0.19	1.33	88
 **14**	1.65 ± 0.36	0.75	91
 **24**	9.56	0.13	58
 **45**	1.59 ± 0.53	0.78	87
 **II**	2.10 ± 0.40	0.59	95
 **11**	0.57 ± 0.10	2.18	72
 **19**	>10	<0.12	20
 **31**	0.90 ± 0.04	1.38	68
 **44**	3.04 ± 1.15	0.41	100

[a] EC_{50} values are compound concentrations maintaining cell viability to 50% of non-challenged cells. Values are mean ± SEM of three independent experiments shown in Figures 1 and 2.
[b] Relative (to **I**) potencies were calculated by [$EC_{50\ \mathbf{I}}/EC_{50\ compound}$].
[c] Compounds are considered to be fully, partially or weakly neuroprotective if % efficacy at 10 μM was respectively, >66–100, >33–66 and ⩽33%; ns = non-significant.

dried and the solvent was evaporated in vacuo. The residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3) to afford **11** as yellow gummy solid. Yield: 0.015 g (53%). ^1H NMR δ: 4.02 (s, 2H), 3.57–3.53 (m, 1H, –C*H*SS–), 3.18–3.11 (m, 2H, –CH_2SS–), 2.84–2.77 (m, 4H, ArCH_2, OC(=N)CH_2–), 2.48–2.42 (m, 1H, –HC*H*CH_2SS–), 2.22 (s, 3H, Ar*Me*), 2.10 (s, 3H, Ar*Me*), 1.93–1.67 (m, 7H), 1.59–1.49 (m, 2H), 1.28 (s, 6H). ^{13}C NMR δ: 179.6, 168.8, 146.2, 145.5, 125.4, 125.3, 118.6, 116.4, 72.6, 56.1, 40.2, 38.5, 34.4, 32.8, 28.6, 26.7, 26.3, 26.0, 23.3, 20.7, 12.5, 12.0. MS *m/z*: 448.5 $(M+H)^+$. HRMS calcd for $C_{23}H_{32}O_3N_2S_2$: 447.1776, found: 447.1760.

5.1.10. *N*-(5-(1,2-Dithiolan-3-yl)pentanoyl)-3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-carbohydrazide (13)

To a solution of 3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-carbohydrazide **12** (0.080 g, 0.3 mmol) in 3 mL anhyd THF, was added a solution of *N*-hydroxysuccinimide activated lipoic acid (0.092 g, 0.3 mmol) in 3 mL anhyd THF the mixture was stirred at rt overnight. The solvent was then evaporated and the residue was diluted in AcOEt. The organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated and the residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 95:5). Yield: 0.120 g (88%), yellow solid mp 114–116 °C. ^1H NMR δ: 8.96 (d, J = 5.7 Hz, 1H), 8.65 (d, J = 5.7 Hz, 1H), 3.56–3.48 (m, 1H), 3.18–3.06 (m, 2H), 2.65–2.57 (m, 2 H), 2.47–2.41 (m, 1H), 2.29–2.23 (m, 2H), 2.20 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 1.95–1.87 (m, 3H), 1.67–1.40 (m, 9H). MS *m/z*: 453.5 $(M+H)^+$.

5.1.11. 5-(4-(1,2-Dithiolan-3-yl)butyl)-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)-1,3,4-oxadiazole (14)

A mixture of 0.025 g of analogue **13**, and $POCl_3$ (0.2 mL) was heated at 100 °C for 1 h. After completion of the reaction cold water was added and the mixture was extracted with CH_2Cl_2, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated and the residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3). Yield: 0.009 g (38%), white gummy solid. ^1H NMR δ: 4.29 (d, J = 4.7 Hz, 1H), 3.52–3.47 (m, 1H), 3.17–3.08 (m, 2H), 2.77 (t, J = 7.4 Hz, 2H) 2.71–2.64 (m, 2H), 2.42–2.38 (m, 1H), 2.16 (s, 3H), 2.15 (s, 3H), 2.05 (s, 3H), 1.89–1.36 (m, 12H). MS *m/z*: 435.2 $(M+H)^+$. HRMS calcd for $C_{22}H_{30}O_2N_3S_2$: 434.1698, found: 434.1671.

5.1.12. (1,2-Dithiolan-3-yl)pentanoyl-hydrazide (16)

To a solution of 1,2-dithiolan-3-yl pentanoic acid ethyl ester **15** (0.110 g, 0.47 mmol) in 3 mL anhyd CH_3OH, were added $NH_2NH_2{\cdot}H_2O$ (0.15 mL) and the mixture was stirred at 40 °C overnight. After completion of the reaction the mixture was extracted with AcOEt, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered and the solvent evaporated. Purification by flash-column chromatography (CH_2Cl_2/CH_3OH 90:10). Yield: 0.070 g (73%), yellow solid, mp 66–68 °C. ^1H NMR δ: 6.80 (br s, 1H), 3.58–3.53 (m, 1H), 3.19–3.08 (m, 3H), 2.48–2.42 (m, 1H), 2.15 (t, J = 7.3 Hz, 2H), 1.95–1.86 (m, 1H), 1.72–1.64 (m, 4H), 1.51–1.42 (m, 2H).

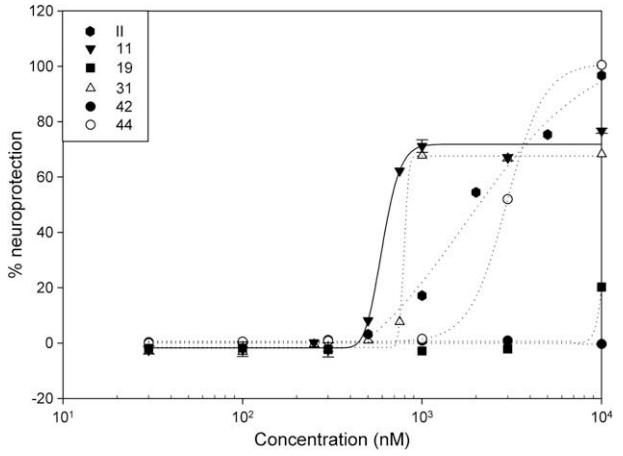


Figure 2. Protection of HT22 cells from oxidative stress-induced cell death by 5-substituted chroman analogues.

5.1.13. *N*-(5-(1,2-Dithiolan-3-yl)pentanoyl)-*N*′-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-carbonyl)-hydrazine (17)

To a solution of 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-carboxylic acid (0.030 g, 0.113 mmol) in 2 mL anhyd DMF, was added at 0 °C, Et_3N (0.1 mL). After 30 min, a solution of BOP (0.050 g, 0.113 mmol) in 1 mL anhyd CH_2Cl_2 and a solution of analogue **16** (0.023 g, 0.113 mmol) in 1 mL anhyd CH_2Cl_2, were added and the mixture was stirred at rt overnight. After completion of the reaction the mixture was extracted with AcOEt, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered and the solvent evaporated and the residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 95:5). Yield: 0.020 g (40%), yellow gummy solid. ^1H NMR δ: 8.86 (d, J = 4.5 Hz, 1H), 8.74 (d, J = 4.5 Hz, 1H), 3.68 (s, 3H), 3.58–3.54 (m, 1H), 3.17–3.09 (m, 2H), 2.85 (t, J = 6.7 Hz, 2H), 2.48–2.39 (m, 1H), 2.34 (t, J = 7.4 Hz, 2H), 2.13 (s, 3H), 2.09 (s, 3H), 1.93–1.84 (m, 1H), 1.73 (t, J = 6.7 Hz, 2H), 1.57–1.37 (m, 6H), 1.29 (s, 6H). ^{13}C NMR δ: 170.2, 165.2, 148.4, 148.3, 129.0, 128.7, 123.3, 117.4, 73.7, 62.5, 56.3, 40.2, 38.5, 34.6, 33.9, 32.5, 29.7, 28.8, 26.9, 25.0, 20.5, 12.3.

5.1.14. 2-(4-(1,2-Dithiolan-3-yl)butyl)-5-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)-1,3,4-oxadiazole (18)

A mixture of 0.012 g of analogue **17**, and $POCl_3$ (0.2 mL) was heated at 100 °C for 1 h. After completion of the reaction cold water was added and the mixture was extracted with CH_2Cl_2, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered, the solvent evaporated and the residue was purified by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3). Yield: 0.008 g (67%), yellow gummy solid. ^1H NMR δ: 3.64 (s, 3H), 3.62–3.58 (m, 1H), 3.10–3.01 (m, 2H), 2.94 (t, J = 6.4 Hz, 2H), 2.68 (t, J = 6.7 Hz, 2H), 2.48–2.40 (m, 1H), 2.21 (s, 3H), 2.16 (s, 3H), 1.95–1.88 (m, 1H), 1.75 (t, J = 6.7 Hz, 2H), 1.57 (m, 6H), 1.32 (s, 6H).^{13}C NMR δ: 150.4, 149.9, 148.3, 139.5, 130.2, 129.0, 118.6, 115.0, 73.7, 62.0, 56.2, 40.2, 38.5, 34.4, 32.3, 29.7, 28.6, 26.9, 25.3, 21.2, 12.5, 12.4. MS *m/z*: 449.5 $(M)^+$. HRMS calcd for $C_{23}H_{32}O_3N_2S_2$: 449.1933, found: 449.1956.

5.1.15. 2-(4-(1,2-Dithiolan-3-yl)butyl)-5-(3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)-1,3,4-oxadiazole (19)

This compound was prepared according to the procedure described for **11** using analogue **18** (0.007 g, 0.016 mmol). Purification by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3). Yield: 0.004 g (60%), yellowish gummy solid. ^1H NMR δ: 3.64–3.57 (m, 1H), 3.20–3.12 (m, 2H), 3.04–2.95 (m, 4H), 2.50–2.46 (m, 1H), 2.25 (s, 3H), 2.19 (s, 3H), 1.96–1.57 (m, 9H), 1.32 (s, 6H). MS *m/z*: 436.4 $(M+H)^+$. HRMS calcd for $C_{22}H_{30}O_3N_2S_2$: 435.1776, found: 435.1755.

5.1.16. General procedure for the preparation of alkynes (23, 27 and 28)

To a solution of aldehyde (1 mmol) in 6 mL anhyd CH_3OH, were added K_2CO_3 (2 mmol) and the Bestmann-Ohira reagent (1.4 mmol) and the mixture was stirred at rt for 24 h. The solvent was then evaporated and the residue was taken up by Et_2O. The organic layer was washed with satd aqueous NaCl, dried with

Na_2SO_4, filtered and the solvent evaporated. Purification by flash-column chromatography (pet. ether/ether, 90:10) afforded the desired compounds.

5.1.16.1. 2-Ethynyl-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-6-benzopyranol (23). This compound was synthesized according to the general procedure for the preparation of alkynes using analogue **22** (0.070 g, 0.3 mmol). Yield: 0.061 g (88%). ^{1}H NMR δ: 3.08–3.03 (m, 1H), 2.85–2.79 (m, 1H), 2.37 (m, 1H), 2.33 (m, 1H), 2.03 (s, 3H), 1.99 (s, 6H), 1.87 (s, 1H), 1.61 (s, 3H).

5.1.16.2. 3,4-Dihydro-5-ethynyl-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran (27). According to the general procedure for the preparation of alkynes using the 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-carboxaldehyde (0.124 g, 0.5 mmol) compound **27** was obtained as white gummy solid. Yield: 0.050 g (41%). 1H NMR δ: 3.80 (s, 3H), 3.43 (s, 1H, ArC≡CH), 2.82 (t, J = 6.8 Hz, 2H), 2.17 (s, 3H), 2.11 (s, 3H), 1.78 (t, J = 6.8 Hz, 2H), 1.30 (s, 6H).

5.1.16.3. 3,4-Dihydro-6-methoxy-5-(prop-2-ynyl)-2,2,7,8-tetramethyl-2*H*-benzopyran (28). This compound was synthesized according to the general procedure for the preparation of alkynes using 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-acetaldehyde (0.100 g, 0.38 mmol).Yield: 0.040 g (41%) yellow gummy solid. ^{1}H NMR δ: 3.74 (s, 3H), 3.54 (d, J = 2.7 Hz, 2H), 2.82 (t, J = 6.8 Hz, 2H), 2.20 (s, 3H), 2.11 (s, 3H), 1.97 (t, J = 2.7 Hz, 1H), 1.83 (t, J = 6.8 Hz, 2H), 1.33 (s, 6H).

5.1.17. General procedure for the preparation of 1,4-substituted 1,2,3-triazoles (24, 29 and 30)

To a solution of the appropriate alkyne (0.1 mmol) in 2 mL *t*-BuOH/H_2O (2:1), were added azide (0.2 mmol) in 1 mL *t*-BuOH/H_2O (2:1), $CuSO_4{\cdot}5H_2O$ (0.03 mmol) and sodium ascorbate (0.06 mmol) and the mixture was stirred at rt for 24 h. AcOEt and aqueous NH_4OH. The organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered and the solvent evaporated. Purification by flash-column chromatography (pet. ether/AcOEt, 50:50) afforded the desired compounds.

5.1.17.1. 1-(5-(1,2-Dithiolan-3-yl)pentyl)-4-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran-2-yl)-1*H*-1,2,3-triazole (24). This compound was synthesized according to the general procedure for the preparation of 1,2,3-triazoles, using analogue **23** (0.015 g, 0.065 mmol) and 3-(5-azidopentyl)-1,2-dithiolane (0.015 g, 0.065 mmol). Yield: 0.012 g (40%). ^{1}H NMR δ: 7.34 (s, 1H), 4.25 (t, J = 7.2 Hz, 2H), 3.58–3.49 (m, 1H), 3.19–3.05 (m, 2H), 2.97 (d, J = 11.7 Hz, 1H), 2.68 (d, J = 11.7 Hz, 1H), 2.47–2.41 (m, 2H), 2.12 (s, 3H), 1.93 (s, 3H), 1.88 (s, 3H), 1.71–1.65 (m, 5H), 1.57 (s, 3H), 1.46–1.33 (m, 5H). HRMS calcd for $C_{23}H_{34}O_2N_3S_2$ $(M+H)^+$: 448.2092, found: 448.2113.

5.1.17.2. 1-(5-(1,2-Dithiolan-3-yl)pentyl)-4-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)-1*H*-1,2,3-triazole (29). The compound was synthesized according to the general procedure for the preparation of 1,2,3-triazoles using analogue **27** (0.022 g, 0.09 mmol) and 3-(5-azidopentyl)-1,2-dithiolane (0.042 g, 0.18 mmol). Yield: 0.010 g (24%) yellow viscous oil. ^{1}H NMR δ: 7.66 (s, 1H, ArC=C*H*–), 4.42 (t, J = 7.1 Hz, 2H), 3.63–3.52 (m, 1H), 3.33 (s, 3H), 3.22–3.09 (m, 2H), 2.74 (t, J = 6.8 Hz, 2H), 2.48–2.42 (m, 1H), 2.20 (s, 3H), 2.14 (s, 3H), 1.92–1.83 (m, 1H), 1.69 (t, J = 6.8 Hz, 2H), 1.67–1.38 (m, 8H), 1.32 (s, 6H). ^{13}C NMR δ: 151.7, 151.5, 149.1, 148.4, 128.1, 127.0, 123.7, 118.4, 73.4, 60.8, 56.3, 53.4, 50.2, 40.2, 38.5, 34.7, 32.9, 30.1, 28.6, 27.0, 26.2, 22.0, 12.5, 12.2. MS *m/z*: 463.4 $(M+H)^+$. HRMS calcd for $C_{24}H_{35}O_2N_3S_2$: 462.2249, found: 462.2249.

5.1.17.3. 1-(5-(1,2-Dithiolan-3-yl)pentyl)-4-((3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1*H*-1,2,3-triazole (30). The compound was synthesized according to the general procedure for the preparation of 1,2,3-triazoles using analogue **28** (0.030 g, 0.12 mmol) and 3-(5-azidopentyl)-1,2-dithiolane (0.055 g, 0.24 mmol). Yield: 0.016 g (28%) yellow viscous oil. ^{1}H NMR δ: 7.07 (s, 1H), 4.22 (t, J = 6.7 Hz, 2H), 4.07 (s, 2H), 3.64 (s, 3H), 3.54–3.50 (m, 1H), 3.17–3.10 (m, 2H), 2.65 (t, J = 6.2 Hz, 2H), 2.47–2.40 (m, 1H), 2.20 (s, 3H), 2.10 (s, 3H), 1.89–1.81 (m, 1H), 1.71 (t, J = 6.2 Hz, 2H), 1.63–1.41 (m, 8H), 1.26 (s, 6H). ^{13}C NMR δ: 149.3, 148.3, 128.2, 127.0, 124.6, 121.4, 121.3, 117.4, 73.0, 61.2, 56.3, 50.1, 40.2, 38.5, 34.6, 32.7, 30.0, 28.6, 26.8, 26.5, 26.2, 23.1, 20.4, 12.9, 12.0. MS *m/z*: 477.5 $(M+H)^+$. HRMS calcd for $C_{25}H_{37}O_2N_3S_2$: 476.2405, found: 476.2402.

5.1.18. 1-(5-(1,2-Dithiolan-3-yl)pentyl)-4-((3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2H-benzopyran-5-yl)methyl)-1H-1,2,3-triazole (31)

This compound was prepared according to the procedure described for **11**, using analogue **30** (0.012 g, 0.025 mmol). Purification by flash-column chromatography (pet. ether/AcOEt, 50:50). Yield: 0.005 g (50%) yellow gummy solid. ^{1}H NMR δ: 7.22 (s, 1H), 5.26 (s, 1H), 4.23 (t, J = 6.7 Hz, 2H) 3.94 (s, 2H), 3.51–3.47 (m, 1H), 3.13–3.06 (m, 2H), 2.69 (t, J = 6.6 Hz, 2H), 2.45–2.35 (m, 1H), 2.17 (s, 3H), 2.05 (s, 3H), 1.85–1.31 (m, 11H), 1.23 (s, 6H). ^{13}C NMR δ: 147.0, 145.7, 145.6, 124.7, 124.3, 121.6, 120.3, 115.3, 72.4, 56.3, 50.3, 40.2, 38.4, 34.6, 33.0, 29.9, 29.6, 28.5, 26.6, 26.2, 22.5, 21.0, 12.4, 11.9. MS *m/z*: 463.4 $(M+H)^+$. HRMS calcd for $C_{24}H_{35}O_2N_3S_2$: 462.2249, found: 462.2221.

5.1.19. 1,2-Dithiolan-3-pentanal (33)

0.170 g of 5-(1,2-dithiolan-3-yl)-pentanol were diluted in anhyd DMSO (0.38 mL, 5.3 mmol). To this solution were added *N,N′*-diisopropylcarbodiimide (0.41 mL, 2.65 mmol) and $Cl_2CHCOOH$ (0.05 mL, 0.53 mmol) and the mixture was stirred at rt for 4 h. After completion of the reaction the mixture was extracted with AcOEt, the organic layer was washed with satd aqueous NaCl, dried with Na_2SO_4, filtered and the solvent evaporated. Purification by flash-column chromatography (pet. ether/AcOEt 80:20) afforded the desired aldehyde. Yield: 0.090 g (54%), yellow oil. ^{1}H NMR δ: 9.74 (s, 1H), 3.56–3.51 (m, 1H), 3.16–3.08 (m, 2H), 2.47–2.41 (m, 2H), 1.89–1.85 (m, 1H), 1.72–1.40 (m, 7H). ^{13}C NMR δ: 202.5, 56.5, 43.9, 40.5, 38.7, 34.9, 29.0, 22.0.

5.1.20. 3-(Hexyn-5-yl)-1,2-dithiolane (34)

This compound was synthesized according to the general procedure for the preparation of alkynes using analogue **33** (0.062 g, 0.32 mmol). Yield: 0.017 g (27%) yellow gummy solid. ^{1}H NMR δ: 3.61–3.56 (m, 1H), 3.20–3.08 (m, 2H), 2.50–2.40 (m, 1H), 2.23–2.20 (m, 2H), 1.98–1.89 (m, 2H), 1.73–1.65 (m, 6H).

5.1.21. 4-(4-(1,2-Dithiolan-3-yl)butyl)-1-((3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1*H*-1,2,3-triazole (35)

The compound was synthesized according to the general procedure for the preparation of 1,2,3-triazoles using analogue **34** (0.010 g, 0.054 mmol) and 3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methylazide (0.030 g, 0.11 mmol). Yield: 0.005 g (21%) yellowish gummy solid. ^{1}H NMR δ: 7.25 (s, 1H), 5.51 (s, 2H), 3.67 (s, 3H), 3.55–3.50 (m, 1H), 3.16–3.08 (m, 2H), 2.69 (t, J = 7.5 Hz, 2H), 2.62 (t, J = 6.5 Hz, 2H), 2.44–2.39 (m, 1H), 2.22 (s, 3H), 2.11 (s, 3H), 1.90–1.81 (m, 1H), 1.74–1.43 (m, 8H), 1.25 (s, 6H).

5.1.22. *N*-((3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-(1,2-dithiolan-3-yl)pentanthioamide (40)

To a solution of *N*-((3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-(1,2-dithiolan-3-yl) pentanamide **38** (0.050 g, 0.114 mmol) in 4 mL anhyd THF, Lawesson's reagent (0.046 g, 0.114 mmol) was added and the mixture was refluxed at 70 °C overnight. After evaporation of the solvent under argon the residue was purified by flash-column chromatography (pet. ether/AcOEt, 60:40). Yield: 0.040 g (78%), yellowish solid mp 96–99 °C. ^{1}H NMR δ: 7.47 (s, 1H), 4.83 (d, J = 4.3 Hz, 2H,), 3.68 (s, 3H), 3.55–3.51 (m, 1H), 3.16–3.09 (m, 2H), 2.74 (t, J = 6.6 Hz, 2H), 2.60 (t, J = 7.3 Hz, 2H), 2.46–2.40 (m, 1H), 2.19 (s, 3H), 2.11 (s, 3H), 1.89–1.79 (m, 1H), 1.77 (t, J = 6.5 Hz, 2H), 1.68–1.42 (m, 6H), 1.29 (s, 6H). ^{13}C NMR δ: 204.1, 150.5, 148.8, 128.7, 127.0, 124.0, 117.9, 73.5, 61.5, 56.6, 46.9, 42.8, 40.4, 38.7, 34.8, 32.8, 29.2, 28.6, 27.1, 20.8, 12.9, 12.3. MS m/z: 453.6 $(M+H)^+$.

5.1.23. 5-(4-(1,2-Dithiolan-3-yl)butyl)-1-((3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1*H*-tetrazole (42)

To a solution of **40** (0.030 g, 0.066 mmol) in 0.5 mL anhyd THF were added diisopropylazodicarboxylate (0.02 mL, 0.1 mmol) and triphenylphosphine (0.026 g, 0.1 mmol) and after stirring for 5 min, trimethylsilylazide (0.02 mL, 0.1 mmol) was added. The reaction mixture was stirred at 40 °C for 3 h and then the solvent was evaporated in vacuo. The residue was purified by flash-column chromatography (pet. ether/AcOEt 50:50). Yield: 0.025 g (83%) white gummy solid. ^{1}H NMR δ: 5.49 (s, 2H), 3.60 (s, 3H), 3.52–3.45 (m, 1H), 3.20–3.11 (m, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.57 (t, J = 6.7 Hz, 2H), 2.46–2.38 (m, 1H), 2.19 (s, 3H), 2.10 (s, 3H), 1.89–1.83 (m, 1H), 1.73 (t, J = 6.7 Hz, 2H), 1.62–1.44 (m, 6H), 1.20 (s, 6H). ^{13}C NMR δ: 155.3, 150.1, 148.9, 128.7, 128.5, 121.0, 118.5, 73.6, 61.9, 56.5, 43.4, 40.4, 38.7, 34.7, 32.6, 29.0, 27.1, 27.0, 23.1, 22.2, 20.6, 13.1, 12.5.

5.1.24. 5-(4-(1,2-Dithiolan-3-yl)butyl)-1-((3,4-dihydro-6-hydroxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)methyl)-1*H*-tetrazole (44)

Prepared according to the procedure described for **11**, using **42** (0.020 g, 0.043 mmol). Purification by flash-column chromatography (pet. ether/AcOEt 50:50). Yield: 0.009 g (42%), yellow gummy solid. ^{1}H NMR δ: 5.41 (s, 2H), 3.53–3.46 (m, 1H), 3.16–3.01 (m, 2H), 2.85 (t, J = 7.5 Hz, 2H), 2.71 (t, J = 6.7 Hz, 2H), 2.42–2.35 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.89–1.77 (m, 2H), 1.73 (t, J = 6.7 Hz, 2H), 1.65–1.42 (m, 5H), 1.20 (s, 6H). MS m/z: 449.4 $(M+H)^+$. HRMS calcd for $C_{22}H_{33}O_2N_4S_2$ $(M+H)^+$: 449.2045, found: 449.2074

5.1.25. *N*-(2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)ethyl)-(1,2-dithiolan-3-yl)pentanamide (39)

To a solution of 2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl) ethylamine (0.120 g, 0.46 mmol) in 3 mL anhyd THF, was added *N*-hydroxysuccinimide activated lipoic acid (0.138 g, 0.46 mmol) in 3 mL anhyd THF and the mixture was stirred at rt for 24 h. The solvent was then evaporated and the residue was taken up by AcOEt. The organic layer was washed with satd aqueous NaCl, dried over Na_2SO_4, filtered and the solvent was evaporated in vacuo. Purification by flash-column chromatography (CH_2Cl_2/CH_3OH 95:5) gave **39** as yellow gummy solid. Yield: 0.110 g (53%). ^{1}H NMR δ: 6.35 (br s, 1H), 3.65 (s, 3H), 3.55–3.50 (m, 1H), 3.36–3.33 (m, 2H), 3.16–3.07 (m, 2H), 2.79 (t, J = 6.5 Hz, 2H), 2.67 (t, J = 6.5 Hz, 2H), 2.45–2.39 (m, 1H), 2.17 (s, 3H), 2.07 (s, 3H), 1.89–1.85 (m, 1H), 1.77 (t, J = 6.7 Hz, 2H), 1.66–1.61 (m, 6H), 1.30 (s, 6H). ^{13}C NMR δ: 173.1, 149.4, 148.5, 127.9, 126.7, 124.5, 117.1, 72.9, 60.8, 56.4, 40.2, 38.4, 36.4, 34.6, 32.8, 28.8, 26.9, 25.6, 25.4, 25.2, 20.2, 12.8, 11.9.

5.1.26. *N*-(2-(3,4-Dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)ethyl)-(1,2-dithiolan-3-yl)pentanethioamide (41)

Prepared from **39** (0.110 g, 0.24 mmol) using the procedure described for **40**. Purification by flash-column chromatography (CH_2Cl_2/CH_3OH 97:3) Yield: 0.080 g (71%), yellow gummy solid ^{1}H NMR δ: 8.67 (br s, 1H), 3.71 (s, 3H), 3.66–3.63 (m, 2H), 3.53–3.50 (m, 1H), 3.16–3.07 (m, 2H), 2.92 (m, 2H), 2.66 (t, J = 6.7 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 2.43–2.37 (m, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 1.88–1.63 (m, 7H), 1.50–1.35 (m, 8H). ^{13}C NMR δ: 204.5, 149.0, 148.7, 128.0, 126.3, 125.0, 116.9, 73.0, 60.9, 60.4, 56.3, 47.5, 46.6, 40.2, 38.4, 34.5, 32.6, 28.3, 26.9, 24.0, 20.3, 12.8, 12.0. MS m/z: 468.4 $(M+H)^+$.

5.1.27. 5-(4-(1,2-Dithiolan-3-yl)butyl)-1-(2-(3,4-dihydro-6-methoxy-2,2,7,8-tetramethyl-2*H*-benzopyran-5-yl)ethyl)-1*H*-tetrazole (43)

To a solution of **41** (0.032 g, 0.07 mmol) in 0.5 mL anhyd THF were added diisopropylazodicarboxylate (0.02 mL, 0.11 mmol) and triphenylphosphine (0.029 g, 0.11 mmol) and after stirring for 5 min trimethylsilylazide (0.02 mL, 0.11 mmol) was added. The reaction mixture was stirred at rt for 5 h and then the solvent was evaporated in vacuo. The residue was purified by flash-column chromatography (pet. ether/AcOEt 50:50). Yield: 0.012 g (36%), white gummy solid. ^{1}H NMR δ: 4.45 (t, J = 6.7 Hz, 2H), 3.65 (s, 3H), 3.53–3.48 (m, 1H), 3.12–3.09 (m, 2H), 3.06 (t, J = 6.7 Hz, 2H), 2.48–2.42 (m, 1H), 2.34 (t, J = 7.7 Hz, 2H), 2.25 (t, J = 6.9 Hz, 2H), 2.16 (s, 3H), 2.05 (s, 3H), 1.89–1.83 (m, 1H), 1.66–1.58 (m, 8H), 1.30 (s, 6H). ^{13}C NMR δ: 155.1, 149.9, 148.4, 128.4, 125.5, 124.3, 117.4, 73.1, 60.7, 56.2, 46.8, 40.2, 38.5, 34.4, 32.5, 29.7, 28.8, 27.5, 26.7, 26.5, 22.3, 20.2, 12.8, 12.1. HRMS calcd for $C_{24}H_{37}O_4N_2S_2$: $(M+H)^+$ 477.2358, found: 477.2340.

5.1.28. *N*-(5-(1,2-Dithiolan-3-yl)pentyl-2-(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-benzopyran carboxamide (45)

Trolox (0.022 g, 0.09 mmol) in 3 mL anhyd THF was activated with CDI (0.016 g, 0.095 mmol) and to this solution, 5-(1,2-dithiolan-3-yl) pentanamine (0.020 g, 0.11 mmol) in 3 mL THF was added and the mixture was stirred at rt overnight. Purification by flash column chromatography (CH_2Cl_2/CH_3OH 95:5) afforded **45** as yellow gummy solid. Yield: 0.025 g (66%). ^{1}H NMR δ: 6.41 (d, J = 4.9 Hz, 1H), 4.52 (br s, 1H), 3.52–3.49 (m, 1H), 3.34–3.23 (m, 1H), 3.19–3.09 (m, 2H), 2.61–2.58 (m, 2H), 2.49–2.38 (m, 2H), 2.20 (s, 6H), 2.11 (s, 3H), 1.94–1.86 (m, 3H), 1.58–1.25 (m, 11H). ^{13}C NMR δ: 174.5, 145.8, 144.6, 122.0, 121.7, 118.4, 118.3, 78.7, 56.8, 40.5, 39.1, 38.8, 35.1, 29.8, 29.6, 26.5, 25.1, 25.0, 20.9, 12.6, 12.2, 12.0. MS m/z: 424.3 $(M+H)^+$. HRMS calcd for $C_{22}H_{33}ON_3S_2$: 423.1902, found: 423.1909.

5.2. Biology

5.2.1. Evaluation of the activity of 2-dithiolane/chroman hybrids against oxidative stress-induced cell death of HT22 hippocampal neurons

The hybrids were tested as previously described[34], with minor modifications. Briefly, HT22 cells were plated in a 96-well flat bottom plate at a density of 4000 cells per well in 100 μl of DMEM-Hepes-GlutaMAX medium containing 10% of fetal bovine serum. 24 h after plating, the cells were challenged with 5 mM glutamate in the absence or presence of increasing concentrations of the hybrids in fresh medium for 24 h prior to assessing the relative numbers of living cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. MTT conversion to coloured formazan was assessed from the difference in optical density (dOD) at 550 and 670 nm. Direct interference of the test compounds with MTT conversion to formazan was excluded using mock cultures de-

prived of HT22 cells. Interference of the hybrids with mitochondrial conversion of MTT to formazan was excluded using the trypan blue exclusion assay to directly determine the number living cells. No challenged cells served to test cytotoxicity at different hybrid concentrations, whereas challenged cells served to assess neuroprotective activity by comparison. Cells exposed only to vehicle (DMSO) or glutamate served as controls. Cell death (CD) in the absence of hybrids was calculated by $CD_{vehicle} = [(dOD_{vehicle} - dOD_{glutamate}) * 100/dOD_{vehicle}$, whereas cell death in their presence was calculated by $CD_{compound} = [(dOD_{compound} - dOD_{compound+glutamate}) * 100/dOD_{compound}$. Neuroprotection (%) was calculated by $[(CD_{vehicle} - CD_{compound}) * 100/CD_{vehicle}$.

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